C-Functional Group Chemistry of Humic Substances and Their Spatial Variation in Soils

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Organic molecules derived from biological processes and the biochemical alteration of plant and animal residue are common in soils and natural aquatic systems and their concentration ranges from <1 ppm to as high as $4x10^5$ ppm. Their composition varies widely with location and origin (e.g. soil, marine), and consists of small chain molecules (e.g. acetate, citrate), organic macromolecules (e.g. proteins), and polyfunctional humic substances (HS)[1]. Of these, humic substances exist at high concentrations, and are stable to biochemical alteration with long lifetime. In addition, HS can form strong complexes with both inorganic and organic contaminants and mineral surfaces, and thus play a major role in geochemical processes[2]. At least for a century, research has been focused on understanding the HS functional group chemistry and the macromolecular structure - the properties of HS that control their behavior in the environment.

Traditionally researchers have been isolating humic substances from soils and water using various methods (e.g. alkalies, resins), and evaluate their functional group chemistry using different techniques such as nuclear magnetic resonance (NMR) spectroscopy, infrared spectroscopy (IR), and pyrolysis[3]. Although these methods have provided important information on the functional groups of HS of different origin, very little is known about their chemistry in their native state[4]. Low C-concentration of several natural samples, and the presence of other interfering elements/molecules of HS and the mineral matter limit the applications of conventional laboratory techniques in *in-situ* characterization of HS functional group chemistry. Hence HS isolation, purification and preconcentration are necessary for their characterization[5]. However, the recent technological developments in soft X-ray spectroscopic tools, and the construction of third generation synchrotron sources, have made the *in-situ* examination of organics possible. In addition, element-specific chemical information for molecules present in water or on mineral surfaces can be obtained under ambient conditions. We have initiated a research program to understand the chemistry of HS in their native state, and to explore the applications of soft X-rays in probing their functional group chemistry and metal complexation patterns. Several soft X-ray beamlines at the ALS (B.L 8.0, 7.0, 6.3.2, 9.3.2) and other complimentary facilities at the Lawrence Berkeley National Laboratory (NCEM), and the Stanford Synchrotron Radiation Laboratory have been used for this study. In this report, the C-functional group chemistry of isolated and pristine HS is discussed here.

EXPERIMENTAL METHODS

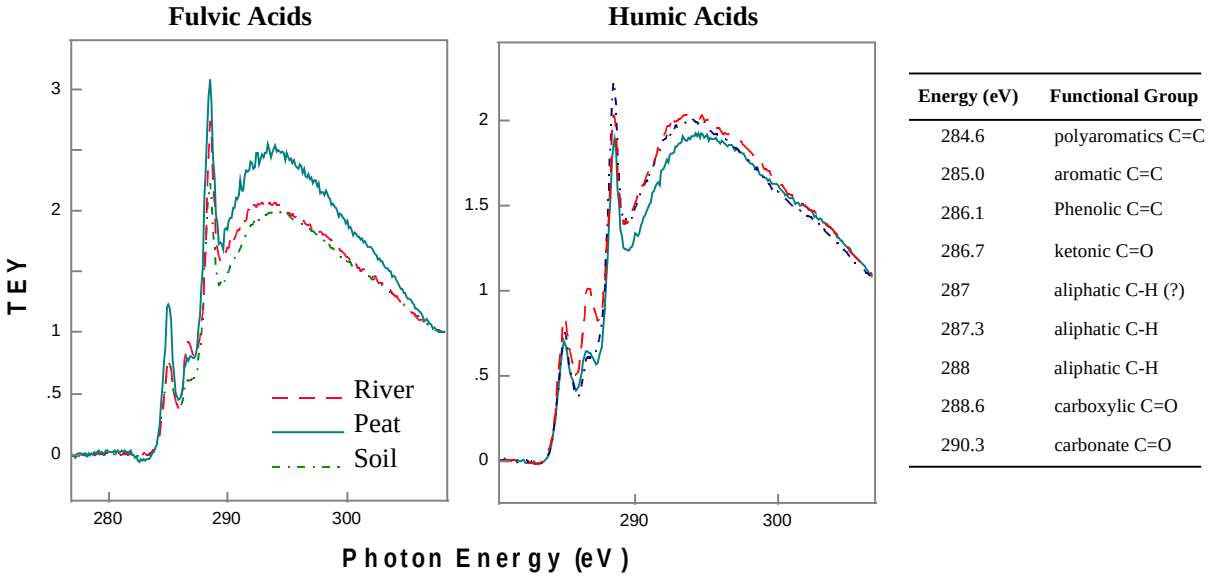
In-situ functional group chemistry of HS of different origin (fluvial, soil, peat) and several other structural models (e.g. carboxylic acids, amino acids) is evaluated using SXEER (soft X-ray Endstation for Environmental Research) on beamline 8.0, and the STXM endstation on beamline 7.0. At this stage of the investigation, we focused on the HS C-NEXAFS features and their relation to the different HS functional groups. In addition, this C-functional group information obtained from the NEXAFS can be compared and correlated with the published NMR data. Excepting for the studies on SXEER (in transmission and fluorescence), the C-NEXAFS spectra

of all aqueous/precipitate samples were collected in transmission mode. Several of these solid samples were also analyzed at beamlines 6.3.2, and 9.3.2 in vacuum (using electron yield detection). For examining liquid samples in transmission mode, liquid droplets/soil suspensions were sandwiched between 1600 A thick windows.

RESULTS AND DISCUSSION

C-Functional Groups of Humic Substances

Previous NMR (C, N, proton) spectroscopic studies of HS indicate that these molecules primarily contain aliphatic C-H, C-N, methoxyl, carbohydrate, carboxyl, alcohol, and ketonic groups, and aromatic C-H, C-N groups[4,5]. The C-NEXAFS spectra of humic substances also exhibit several sharp peaks below 291 eV, which correspond to the $1s \rightarrow \pi^*$ and $1s \rightarrow \sigma^*$ transitions of different C moieties in humics. Since the energies of these electronic transitions are characteristic of different functional groups[6], several HS functional groups can be identified from their C-NEXAFS spectral features (Fig. 1). The spectral region above 291 eV is broad without any sharp features, which may be due to the overlap of broad peaks corresponding to the $1s \rightarrow \sigma^*$ transitions of several HS functional groups. Humic substances isolated from different sources indicate that they contain the same functional groups, but at different concentrations. For instance, the fulvic acids contain high carboxylic to aromatic carbon when compared to the humic acids, and these results are in agreement with that of NMR studies. Although peak intensities in the NEXAFS spectra vary with the orientation of molecules on substrate surfaces[6], the HS in powdered form and in aqueous solutions do not exhibit any specific orientation, and hence their peak intensities can be used to obtain the relative concentrations of different functional groups.



Energy (eV)	Functional Group
284.6	polyaromatics C=C
285.0	aromatic C=C
286.1	Phenolic C=C
286.7	ketonic C=O
287	aliphatic C-H (?)
287.3	aliphatic C-H
288	aliphatic C-H
288.6	carboxylic C=O
290.3	carbonate C=O

Figure 1. C-NEXAFS spectra of fulvic and humic acids. The peak positions shown in the table (on right) are obtained from curve-fitting. The peaks corresponding to cyanide groups overlap with those of C=C, and C=O and hence they can not be interpreted unambiguously from the C-NEXAFS spectra of the mixture. However, the N-NEXAFS spectra of HS can provide that complimentary information.

Our studies also indicated that the NEXAFS spectra of air dried HS collected in vacuum and at atmospheric pressure conditions exhibit the same spectral features, which suggests that vacuum conditions do not alter the chemistry of solid HS. However, aqueous humic substances exhibit significant changes in the spectral features of HS functional groups due to their protonation,

changes in molecular conformation, and/or metal complexation. Although the total number of C-functional groups that can be identified by the NMR are greater than that of C-NEXAFS, some of the functional group information can only be obtained from the NEXAFS alone (e.g. on polyaromatics). Unlike the NMR signal, the C-NEXAFS features do not have interference from other elements in soils. In addition, more (either new or complimentary) information can be obtained from the N-, O-, P-, and S-NEXAFS spectral features. Altogether the NEXAFS features of these different functional groups can offer significantly more information on humics in their native state, and for their metal/mineral complexed HS than the other spectroscopic techniques.

Spatial Heterogeneity of Humic Substances in Soils

As mentioned earlier, HS are commonly isolated from soils and aquatic systems using various techniques, before they are analyzed. Otherwise the C concentration of original samples is not sufficient enough for most of the spectroscopic methods. In addition, other elements and soil minerals that associate with HS can interfere with the HS analysis[e.g.7]. To evaluate the influence of isolation procedures on the functional group chemistry of humic substances, we examined the C-functional groups of a pine ultisol (collected from Puerto Rico, USA), and the HS isolated from this soil sample. The C-NEXAFS spectra of organic molecules present in this soil sample is collected from different locations in the sample.

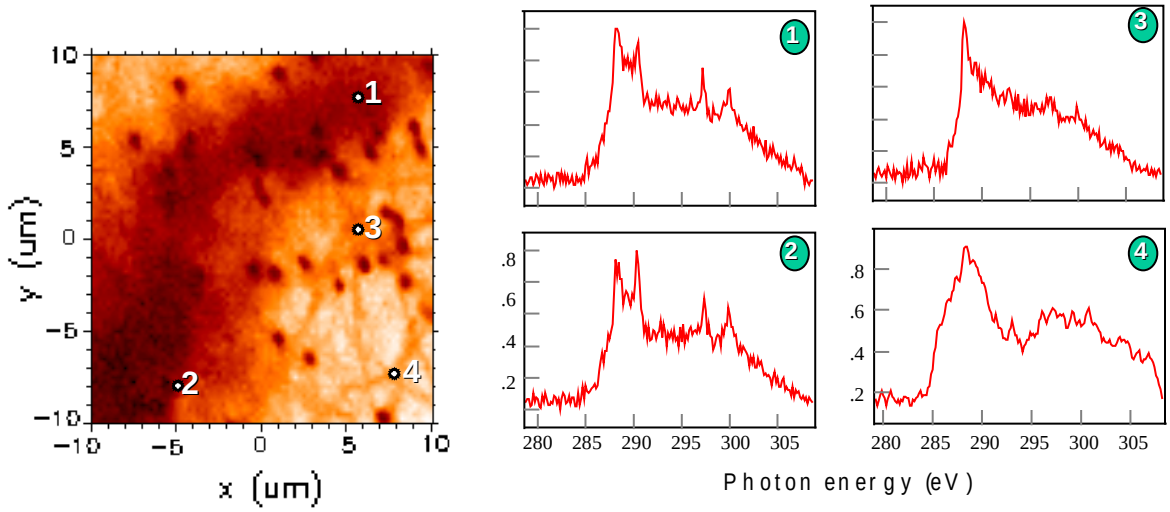


Figure 2. Spatial heterogeneity of C in pine ultisol. The picture on left shows a transmission X-ray microscopy image of soil aggregates in pine ultisol. The soil sample contains Fe-oxides and clays, and the organic carbon concentration in soil is about 4.5 % as C, and a pH of 5.0. The NEXAFS spectra shown on the right are for different locations in the soil aggregates. The sharp peaks at ~ 288.5, 290.5, and the doublet at 300 eV correspond to the carboxylic, carbonate and the potassium L_2 & L_3 edges, respectively. The low-energy shoulder at 287 eV corresponds to the ketonic, and aliphatic C-H groups.

When compared with the NEXAFS spectra of isolated soil humic substances (Fig. 1), pristine soil organic molecules (i.e. soil organics not subjected to isolation procedures) exhibit spectral features representative of carboxylic, carbonate, and ketonic groups with no distinct spectral features of aromatic C. Only one (location 4 in Fig. 2) of the several locations in the soil sample examined (10 µ x 10µ area) showed a broad feature around 285 eV in the C-NEXAFS spectrum, which may correspond to the aromatic C. This spectrum is noisy, which may be because of high sample thickness and/or low C-concentration. However, based on these results one can conclude that carboxylic, carbonate (inorganic, organic) and ketonic groups predominate the C-NEXAFS spectrum of pine ultisol, and the aromatic content is at extremely low concentration. This may

suggest that the HS isolation procedures may be causing the enrichment of organic molecules rich in aromatic unsaturated C, relative to the carboxylic and other groups.

The concentration of organic molecules in aquatic systems are typically very low (< 50 ppm), and it is not feasible to obtain C-NEXAFS spectra in transmission or fluorescence mode for these samples. We are currently testing different solid state detectors to improve the sensitivity of fluorescence detection of dilute aqueous samples. However, high fluorescence yield at high energies permitted the examination of S-functional groups of organics present in fluvial samples and their HS isolates at the SSRL. These studies also indicate that the isolation procedures modify the sample chemistry. Experiments are in progress to characterize the *in-situ* fractionation of metals and contaminants into HS, and their specific interactions with different functional groups of HS.

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